RAPPORT DE MISSION EN INDE CONGRES INTERNATIONAL DE PHYSIOLOGIE VEGETALE

15-20 Février 1988 NEW DELHI

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J.-L. JACOB

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CHRONOLOGIE DE LA MISSION

Dimanche 14 février

Départ Paris 12 heures Arrivée New Delhi 15.02.88 1h15 (heure locale)

Lundi 15 février

Début du Congrès 9h30-12h30 Cérémonie d'ouverture avec le Président de la République et Conférences plénières 14h00-17h45 Conférences (Exposé I.R.CA.) Session 4

Mardi 16 février

9h00-13h00 Session 7 14h00-17h45 Session 7 18h00-20h30 Réception protocolaire

Mercredi 17 février

9h00-13h00 Session 8 14h00-17h45 Session 14

Jeudi 18 février

9h00-13h00 Session 6 14h00-17h45 Session 15

Vendredi 19 février

9h00-13h00 Session 9 14h00-17h45 Session 18

Samedi 20 février

9h00-13h00 Conférence Générale, Session de clôture

Dimanche 21 février

Départ New Delhi 1h00 (heure locale) Arrivée Paris 6h00 Ce congrès très bien organisé a réuni 600 chercheurs de 35 pays différents. Un grand nombre de sujets ont été abordés dont certains m'ont particulièrement intéressé. Le document regroupant les résumés est à disposition au laboratoire de Physiologie-Biochimie à Montpellier. Il y aura vraisemblablement l'édition d'un proceeding rassemblant les interventions dans leur totalité. Il sera dans ce cas intéressant de se le procurer (le titre et la liste des différentes sessions se trouvent en annexe).

En outre j'ai pu prendre contact avec les physiologistes du Rubber Research Institute of India (R.R.I.I.) et avec le Docteur Sethuraj, Directeur de l'Institut.

J'ai également rencontré le Dr Pakianathan du Rubber Research Institute of Malaysia (R.R.I.M.).

Il m'a enfin été possible d'avoir des discussions utiles et des échanges d'idées et d'information avec le Professeur Hess de Stuttgardt (R.F.A.) sur la biotechnologie, avec le Professeur Shina de New Dehli en agrophysiologie, avec le Professeur Dua de Chandigarh (Pendjab) sur la compartimentation cellulaire, avec le Dr Miszalski de Cracovie sur la photosynthèse.

Beaucoup d'étudiants sur le point de terminer leur PhD cherchent des postes à l'étranger pour leur spécialisation post-universitaire. Ils ont pour ce faire des bourses annuelles de l'état dont le montant est d'environ 7 000 U\$. Plusieurs m'ont demandé s'il était possible d'aller en France dans ce but.

En ce qui concerne l'Hevea quatre communications ont été présentées : une par l'I.R.CA, une par le R.R.I.M., deux par le R.R.I.I. Notre publication était intitulée : "LATEX FLOW CELLULAR REGENERATION AND YIELD OF *HEVEA BRASILIENSIS*. INFLUENCE OF THE STIMULATION" (le texte est en annexe).

Le Dr Pakianathan du R.R.I.M. cosignataire avec le Dr P.D. Abraham a présenté un travail intitulé : "PHYSIOLOGICAL BASIS FOR RATIONAL EXPLOITATION OF HEVEA YIELDS". Les résultats portent sur l'analyse des aires drainées en fonction des longueurs d'encoches et de l'utilisation de la stimulation. La photocopie de cet article est en annexe.

Le R.R.I.I. avait deux communications. La première intitulée "ROLE OF PHYSIOLOGICAL INVESTIGATION OF YIELD IMPROVMENT IN *HEVEA BRASILIENSIS*" a été faite par le Dr Sethuraj lui-même. Reprise de nombreuses conférences antérieures, elle présentait cependant quelque intérêt. J'aurai voulu en avoir un exemplaire hélas l'auteur ne l'avait pas mis au propre...il me l'enverra (peut être). Le résumé est en annexe.

La seconde intitulée "STUDIES ON SOIL-HEVEA-ATMOSPHERE SYSTEM :WATER RELATIONS" était signée par G. Gururaja Rao *et al.* Les résultats exposés sont extrêmements intéressants. Les auteurs n'ayant pas encore rédigé l'exemplaire pour le proceeding je n'ai pu faire de photocopies. J'ai toutefois bon espoir de recevoir un exemplaire en avril. Il nous sera très utile dans l'approche nouvelle que nous engageons dans l'axe eau-sol-Hevea-atmosphère. Le résumé est en annexe.

Rencontre avec le Dr Sethuraj

Nous avons abordé avec le Dr Sethuraj les sujets suivants :

- Les prochaines réunions I.R.R.D.B. et le meeting Physiologie-Exploitation à Paris en octobre ou novembre. Le Dr Sethuraj souhaite vivement pouvoir y participer. Cependant la situation économique actuelle en Inde nécessite des contraintes économiques. Il faut, pour que son voyage puisse être financé, qu'il présente des raisons valables. A cet effet il souhaite que, par le canal de Monsieur de Padirac, lui soit envoyé une demande pressante de participation eu égard à sa fonction de Directeur d'Institut dans le cadre de l'I.R.R.D.B. et à sa fonction de coordinateur gérant les actions thématiques de physiologie soutenues par les organismes internationaux, (ATP) en ce qui concerne l'étude des problèmes de l'Heveaculture en zone marginale.

- La coopération I.R.CA.CIRAD-I.R.R.I.I. Le Dr Sethuraj a déjà pris contact avec l'Ambassade de France, et il souscrit a priori à toutes les formes de coopération qui pourraient être négociées. Mais il est nécessaire que la démarche parte de Paris. Dans ce cadre l'envoi d'un chercheur physiologiste de l'I.R.CA. pour un temps déterminé pourrait peut être envisagée. Elle permettrait d'initier une nouvelle approche de recherche au plan notamment des problèmes des relations eau-sol-plante et des problèmes inhérents à la culture de l'Hevea en zone marginale.

- L'Heveaculture en zone marginale. Le R.R.I.I. a une base d'une vingtaine de chercheurs dans le Nord-Est de l'Inde à Kunjaban (Agartala) et neuf points d'expérimentations. J'ai demandé des informations sur les conditions écoclimatiques de cette région ainsi que sur l'Heveaculture qui y est pratiquée.

J'ai également fait la même demande au Dr D. Choudhuri, qui est physiologiste dans ce centre. Nous devrions normalement recevoir ces documents dans un proche avenir.

CONTENTS

Overview Session		1
1.	Physiological Basis of Yield : Cereals	5
2.	Physiological Basis of Yield : Grain Legumes	25
3.	Physiological Basis of Yield: Oilseeds	41
4.	Physiological Basis of Yield : Plantation, Tuber and Fibre Crops	53
5.	Modelling : Plant Processes	67
6.	Photosynthesis	76
7.	Plant Molecular Biology and Genetic Engineering	93
8.	Plant Growth Regulators : Molecular Biology	105
9.	Water Stress and Crop Productivity	125
10.	Light and Temperature Stresses	155
11.	Salinity Stress	167
12.	Nutrition : Nitrogen	187
13.	Nutrition : Iron	203
14.	Nutrition : Micronutrients \ldots	213
15.	Nutrition : Phosphorus and Others \ldots \ldots \ldots \ldots \ldots	225
16.	Seed Physiology and Biochemistry	243.
17.	Physiology of Reproduction	261
18.	Pre- and Post-Harvest Physiology	· 273

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4.1 ROLE OF PHYSIOLOGICAL INVESTIGATIONS ON YIELD IMPROVEMENT IN HEVEA BRASILIENSIS

M. R. Sethuraj

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The Para Rubber tree—*Hevea brasiliensis*—is the major source of natural rubber. Rubber is obtained by processing the latex collected by wounding the bark of the tree. The flow of latex is an abnormal physiological phenomenon induced by tapping. The yield obtained in a days' tapping thus depends on the volume of latex collected and the rubber content. The cumulative yield over a period of time, however, depends upon the biomass increment and partitioning into economic yield. Studies on major and minor yield components have contributed substantially to characterise high yielders and low yielders and to use such information in plant improvement programmes. Decades of research on exploitation systems have standardised the procedure of latex extraction keeping in view the optimum physiological balance. Chemical methods for enhancing latex flow also have been identified. Physiological investigations are also important in involving early prediction methods for potential yield and stress tolerance.

9.15 STUDIES ON SOIL-HEVEA-ATMOSPHERE SYSTEM : WATER RELATIONS

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Components of Soil-hevea-Atmosphere system were examined in four clones during the moisture-stress period in 1987. Semidiurnal variations in plant and atmospheric factors were recorded. Results indicate that the pre-dawn leaf water potentials (ψ_1) were almost similar in all the clones and decreased during the day. Stomatal resistance was minimum at 10.00 h and increased in the afternoon. Transpiration (Tn) rates were maximum at 10.00 h. Maximum xylem sap speed (xss) was noticed around midday. Higher rates of xss even when the Tn is decreased indicate a phenomenon of water recoupment lost in the plant system. Soil moisture was found to be below the wilting point up to 60 cm depth and higher pre-dawn ψ_1 indicate that plants are extracting water from the deeper layers of soil. The latex vessel turgor prior to tapping was found to be equal to the calculated values using pre-dawn ψ_1 and latex solute potentials. Positive turgor was maintained throughout the day including the period of latex flow. Tapping resulted in a sudden drop in latex vessel turgor. Rebuilding of turgor was noticed with the initiation of latex vessel plugging. Turgor changes were found to be associated with the changes in latex solute potentials.

LATEX FLOW, CELLULAR REGENERATION AND YIELD OF HEVEA BRASILIENSIS INFLUENCE OF HORMONAL STIMULATION

by J.L. JACOB*, J.C. PREVOT*, J.M. ESCHBACH**, R. LACROTTE**, E. SERRES** and A. VIDAL*

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SUMMARY

The latex of *Hevea brasiliensis* is a cytoplasm. Its production depends on two limiting factors ; its flow, and its *in situ* reconstitution.

Some physiological parameters of latex are linked with one and (or) the other of these two factors. Such is the case for dry content, redox potential, sucrose, Pi and RSH contents, total phosphatasic acid activity and the lutoids (vacuolysosomal compartment) stability. The study of the variations of these parameters allows the understanding of the regeneration processes and of the mechanisms involved in latex yield. Its permits also to shed light on the phenomena induced by ethylene treatments used for production stimulation, treatments which greatly modify the laticiferous vessels physiology (hydric, ionic, organic-membrane transports, proteosynthesis, energetic metabolism, homeostasis...). From an applied point of view the knowledge of these parameters and of their physiological signification, can help to optimise exploitation by using adequate tapping and stimulation intensity.

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The productive laticiferous system in *Hevea* bark consists of concentric monocellular rings created from the cambium and which are differentiated in the secondary phloem. Each of these layers or laticiferous rings is formed by anastomosed cells which make up a true paracirculatory system (DICKENSON, 1964; HEBANT, DE FAYE, 1980; GOMEZ, 1982; D'AUZAC, JACOB and CHRESTIN, 1988). When these paracirculatory systems are sectioned during tapping, the turgor pressure in the cortex tissue expels their contents : latex (SOUTHORN, 1969; SETHURAJ and RAGHAVENDRA, 1987). Latex, which contains rubber is therefore a true cytoplasm (DICKENSON, 1964; HEBANT, DE FAYE, 1980).

The production of *Hevea brasiliensis* depends partly on flow (the longer and easier the flow the more latex will be collected) and partly on regeneration which must compensate effectively for the loss of cell material during tapping before the next tapping. The time between two tappings varies and may differ considerably between different cases and different countries.

The main driving force behind latex flow is turgor pressure, but hydrous transfer from neighbouring tissue to the laticifers is also involved. Flow is stopped by complex mechanisms which cause the flocculation of rubber particles by modifying the electro-negative charges which characterise their membranes and which are responsible for the colloidal stability of latex (SOUTHORN, 1969). This is followed by coagulation which leads to the plugging of the wound.

Analysis of certain biological parameters of this cytoplasm (latex) makes it possible to evaluate the physiological state of the laticiferous system (ESCHBACH et al. 1984, JACOB et al. 1985).

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MATERIAL AND METHODS

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Trees of clone GT 1 were planted in the same plot in 1973 in Ivory Coast. They were opened at 1.20 m in 1979 and tapped in a half-spiral twice a week. 72 trees were selected for their homogeneous production.

Tapping was carried out at six different intervals : 1, 2, 4, 7, 14, and 21 days. Twelve trees subjected to each treatment were analysed individually. The different treatments were continued for two months when all the treatments were tapped on the same day and the following analyses carried out : production, total solids content, sucrose, redox potential, acid phosphatase and bursting index. Assays were carried out using methods which have been described elsewhere (ESCHBACH *et al.* 1984).

Sampling of latex for analyses of inorganic phosphate and thiols (RSH) was carried out on trees tapped at 7-day intervals by needle-tapping every 12 hours 10 cm below the tapping out using techniques already described (SERRES, unpublished data).

For ethrel treatment trees (GT 1 clone) trees were treated with a oil palm mixture containing 5 % to 10 % of active matter (100 mg of chloroethylphosphonic acid by tree and by treatment). The trees tapped -every 3 days the week are stimulated 4 times per year -every seven days ten times per year -and the others ones before each tapping (ESCHBACH, 1986).

RESULTS

In addition to production, the various latex parameters were : sucrose content, total solids content (TSC), total acid phosphatase (TAP), bursting index (BI), redox potential (RP), thiols content (RSH) and inorganic phosphate content (Pi). Their physiological significance has been analysed in previous work (ESCHBACH et al. 1984, JACOB et al. 1985, PREVOT et al. 1986).

The involvement of these parameters in regeneration and/or flow mechanisms is summarised and shown in schematic form in table 1. Figure 1 and 2 show the evolution of these parameters in function of the latency

between two tappings in GT 1 trees which were not stimulated with Ethrel.

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Production per tapping per tree was low when trees were tapped every day. Production increased when the interval between two tappings was longer; it reached maximum in trees tapped every 4 days to 7 days but fell considerably when they were tapped only every 14 days and even more so when they were tapped every 21 days.

The first part of this curve is easily accounted for. It means that intralaticiferous regeneration is incomplete between two tappings, resulting in a negative effect on production of trees tapped too often. This hypothesis was confirmed by examination of evolution of the other parameters under the same conditions.

Total solids content (TSC) : it should be remembered that this consists of over 90 % cis-polyisoprene (COMPAGNON, 1986) and that it reflects the regeneration of the contents of the laticifers particularly with regard to rubber.

Figure 1 shows effectively that it is necessary to wait for 7 days before this parameter reaches a value approaching 50 % and then peaks out, which is a sign of metabolic slowing.

Sucrose is the initiatory molecule in rubber synthesis and also of cellular energy. Sucrose content was very low in trees tapped every day. This shows that the molecule is used rapidly and that unavailability can become a limiting factor for production (TUPY, 1973b, ESCHBACH *et al.* 1984, JACOB *et al.* 1985). The sucrose content increases with the interval between two tappings. In this case there is a better balance between the supply of the carbohydrate, which is essential for latex regeneration, and its *in situ* metabolism.

The strong increase in the amount in latex from trees tapped every 7 days and onwards indicates a slowing of use and therefore a slowing of the metabolism of the laticiferous tissue. -5-

The redox potential reflects the metabolic conditions in latex (PREVOT, JACOB, VIDAL, 1984). More negative values indicate the medium is favorable for anabolic phenomena and active synthesis reactions. Figure 1 shows that the redox potential in latex from trees tapped frequently (interval of 1 to 4 days) is low, but at longer intervals it rises and is a sign of a slowing down of the metabolism.

Acid phosphatases which are present in the lutoids, provide information about the vacuolysomal aspect of latex (PUJARNISCLE, 1968). These particles play an essential role in the metabolism of this cell type (RIBAILLIER, JACOB, D'AUZAC, 1971; CHRESTIN, 1984; CHRESTIN *et al.* 1984 a,b) and in tapping flow (SOUTHORN, 1969). Reconstitution of total acid phosphatases (TAP), which reflects that of lutoids, is very rapid between two tappings ; it reaches a peak two days after tapping but it incomplete when tapping is carried out every day.

The bursting index reports on the state of the lutoids since it is measured in percentage of free acid phosphatase in relation to total acid phosphatase. These activities were measured with and without addition of cationic product (Triton X 100), which destroys all subcellular particles membranes (RIBAILLIER, JACOB, D'AUZAC, 1971). The state of lutoids is important because it reflects the state of compartmentation of the laticiferous cells and hence their state of functioning, and also the colloidal stability of latex since lutoid serum is a major factor in the mechanisms which stop tapping flow (SOUTHORN, 1969; I-1 ANOWER, BRZOZOWSKA, LIORET, 1975; BRZOZOWSKA-HANOWER *et al.* 1979; CRETIN, BANGRATZ, 1983). The parameter did not vary very much, although it tended to increase slightly. The colloīdal stability was not modified so much.

R-SH are involved in reactions which combat peroxydative phenomena caused by stress and in particular by tapping. They trap the toxic oxygen produced by the activation of lutoid enzyme NADH quinone reductase (D'AUZAC, SANIER, CHRESTIN, 1985) by oxidation into R-SS-R. A low R-SH value one day after tapping is the sign of activation of the functioning of this enzyme, producing toxic oxygen. The phenomenon subsequently dwindles, as proved by the increase in RSH contents. Too frequent tapping is therefore dangerous since it causes senescent phenomena which can lead to serious physiological consequences and cellular degeneration in the form of dry bark phenomena (CHRESTIN *et al.* 1984a, CHRESTIN, 1985).

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Inorganic phosphorus (Pi) reflects metabolic activity. A high value shows that the laticiferous system is very active. Regeneration of rubber releases a great deal of pyrophosphate (LYNEN 1969) which is then hydrolysed by a pyrophosphatase (JACOB, PREVOT, VIDAL, 1986) and by a pyrophosphatefructose-6-phosphate phosphotransferase (PP-PFK) (PREVOT *et al.* 1987). Figure 2 shows that Pi increased during the first 4 days after tapping ; this can be connected with the increase in TSC and hence with the rubber produced ; it then decreased, indicating a slowing of this activity.

The second part of the production curve shows considerable reduction in function of the interval between two tappings when this interval reaches 14 days and more. Several hypotheses can be put forward to account for this phenomenon. The first involves an increase in latex viscosity caused by an increase in TSC ; this may sometimes hinder and limit flow (HANOWER *et al.* 1980 ; JACOB *et al.* 1985). It is nevertheless not very satisfactory in this case since latex TSC values of the trees tapped every 7 days or at greater intervals are not very different and cannot account for the decrease in production observed.

The second hypothesis might involve destabilisation of latex which could be reflected by the bursting index. Very highly significant negative relations have been shown between this parameter and production (RIBAILLIER, 1972; ESCHBACH et al. 1984).

Nevertheless, if the BI is slightly increasing, this does not explain the production decreasing just by itself.

The third hypothesis concerns the availability of biochemical energy in the laticiferous system. In effect, intercellular transport of the various elements involved in particular in the osmoticum of laticiferous cells, and which are concerned in movement of water towards the latter, require energy. This problem is well known in the loading of phloem and in the activ K^+ influx, important osmoticum element, for stomata opening. In all the cases the functioning of a plasmalemmic ATPase creates a proton gradient wint a proton motrice force which permits a H⁺-sucrose co-transport and a K⁺ influx by a facilitated diffusion phenomenon (GIAQUINTA, 1977; MALEK and BAKER, 1978; DELROT and BONNEMAIN, 1979; MARTIN and KOMOR, 1980; KOMOR, 1983). These phenomena have been studied and described with Hevea laticiferous system (LACROTTE, VAN DE SYPE, CHRESTIN, 1985; JACOB *et al.* 1985, LACROTTE unpublished data)

Schematically, in addition to turgor pressure, flow during tapping must depend on movement of solutes and water from tissue around the laticifers into the latter. This water transport within laticifers involves that this medium maintains a sufficiently negative osmoticum potential. Then an active pumping of solutes (as sucrose and K^+) is needed but this pumping depends on biochem cal energy from actives cellular catabolism. In other words, an active, energy-producing mechanism is a major and necessary factor in good flow. On the other hand, if the metabolism is not sufficiently active in the laticifers, flow will be more difficult and production will therefore diminish. The results show that trees tapped every 7 days or at longer intervals displayed a decrease in the metabolic activity of their latex (Figures 1 and 2). The TSC hardly increased any more, which is a sign of the halting of isoprenic synthesis. The sugar content increased considerably since utilisation was less active, and the carbohydrate supply to the laticifers decreased.

Inorganic phosphorous, reflecting the metabolic turnover, is in low concentration. The redox-potential increased, proof that the anabolic processes were decreasing in the medium.

This hypothesis which connects the biochemical energy available in the laticifers and production by means of ease of flow at tapping has been supported by experiments using stimulation with Ethrel, an ethylene generator. In effect, the results of this treatment which has been studied by numerous authors, shows that it induces activation of the metabolism of the laticifers. The sugar content increases (sink effect) (TUPY, 1969), as does

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utilisation because of an increase in the pH (TUPY, 1973a ; TUPY and PRIMOT, 1976; COUPE, 1977; CRETIN *et al.* 1980). The Pi content increases (ESCHBACH *et al.* 1984) as does the RNA content, (COUPE, 1977), and the ATP content (CHRESTIN, 1984).

Figure 3 shows that trees of the same clone (GT 1) stimulated with Ethrel display a production curve with no falling off in function of the interval between tappings, unlike Figure 1. Acceleration of the metabolism of their laticifers caused by ethylene, and hence the increase in the biochemical energy available, caused the fall in production observed previously to disappear. In addition, the time required with half-spiral tapping (1/2 S) for the contents of the laticifers to be regenerated increased to 14 days. With a full spiral (S) where the drainage area is much more extensive because the wound is longer, regeneration time was not attained after 21 days.

In stimulated trees, the major limiting factor is therefore regeneration, since the limiting factor connected with flow has been removed.

DISCUSSION

Production of *Hevea brasiliensis* depends on the duration of flow at tapping and on the regeneration of latex between two tappings.

Flow is very probably linked with processes which require biochemical energy produced through the metabolism. An active metabolism which generates available intracellular energy allows easy flow and consequently high production. This is the case for examples of clone PB 235 *Heveas* (PREVOT *et al.* 1986). If the metabolism is slow or decreases, for example when the interval between two tappings is too long, little biochemical energy is available and flow becomes a limiting factor for production. Action must then be taken, in the form of stimulation, to activate the metabolism and remove the limiting aspect of flow. In this case, the regeneration process between two tappings is of major importance. The experiments carried out clearly show that the interval between two tappings should be long enough to allow intralaticiferous regeneration not to be a limiting factor in production. Too frequent tapping, and daily tapping in particular, causes phenomena of repeated stress causing toxic reactions (appearance of O_2^-) and reflected by a clear decrease in R-SH, incomplete reconstitution of the vacuolysosomal apparatus whose role is functionally primordial, notably in homeostasis of the laticiferous system (CHRESTIN, 1984) and finally, by exhaustion of the reserves of carbohydrate which are indispensable in regeneration of latex. The result is degeneration of the production tissue in *Hevea* taking the form of the bark dryness syndrome (DE FAY, 1985; VAN DE SYPE, 1984).

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These results are of great importance at a practical level, particularly for the optimisation of exploitation of the rubber tree. Reduction in tapping frequency enables the laticiferous system to regenerate its cellular content more thoroughly by avoiding on the one hand the exhaustion of carbohydrate reserves and, on the other, exhaustion of internal protectors (R-SH) which are used too rapidly because of increased tapping stress. The result is an improvement of the physiological state at cell level likely to better express production potential with less risk. In addition, this reduction in frequency makes it possible to "economise" the tapping panel, which is not used so often, and consequently to extend the economic life of the tree, which is an essential point in rubber culture.

Nevertheless, reducing the tapping frequency generally requires the use of a substance, such as ethylene generators in order to activate the laticiferous metabolism and so to facilitate latex flow and consequently to increase production. If carried out with a certain amount of caution, particularly as regards the concentration of the active ingredient (VAN DE SYPE, 1984; ESCHBACH, TONNELIER, 1984; CHRESTIN, 1985), use of these substances does not have any major drawbacks. This technique of optimising production by reducing tapping frequency combined with hormonal stimulation has been clearly demonstrated experimentally and has already given excellent industrial results (ESCHBACH, BANCHI, 1985; ESCHBACH, 1986). It should become a means of progress of considerable importance for world rubber culture within a well-designed socio-economic framework. REFERENCES

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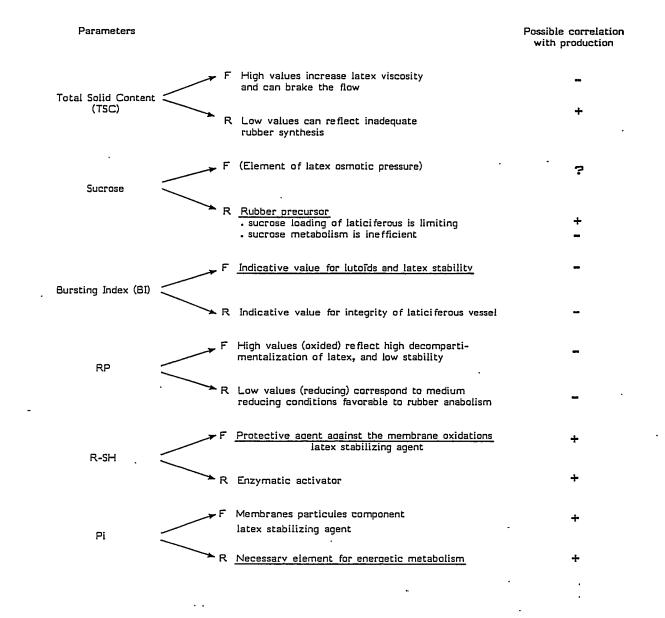
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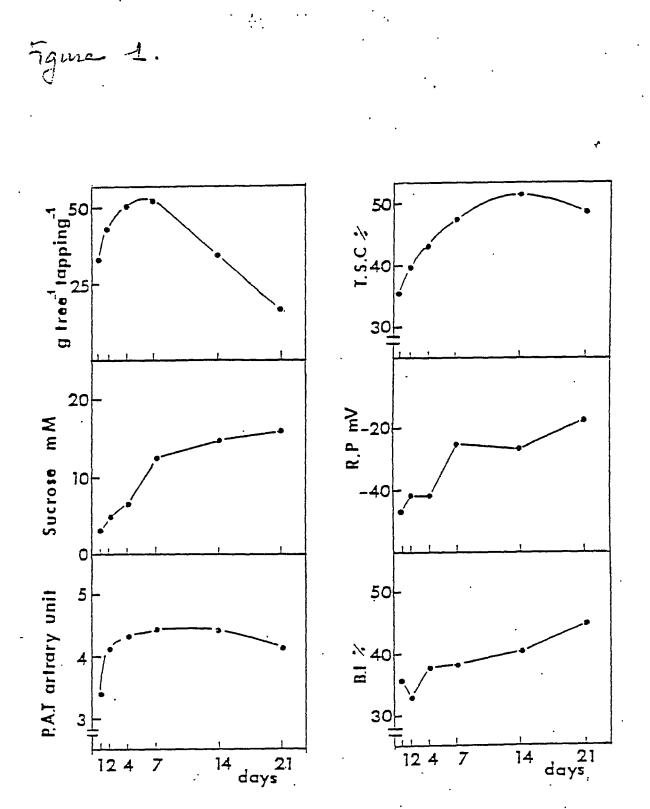
TABLE 1. Physiological parameters of latex and their relation with the production mechanisms: flow (F) and, or regeneration (R)



LEGENDS OF FIGURES

- Figure 1: Evolution of yield and of some latex physiological parameters according to the different time intervals between two tappings. The rubber trees studied (GT1 clone) were not Ethrel treated.
- Figure 2: Evolution of inorganic phosphorus and thiols contents according to the time after tapping. The rubber trees (GT1 clone) are not Ethrel treated and are tapped once a week.
- Figure 3: Yield evolution according to different time intervals between two tappings. The rubber trees (GT1 clone) are tapped with half spiral (1/2 S) or full spiral (S) and Ethrel treated (cf. material and methods).

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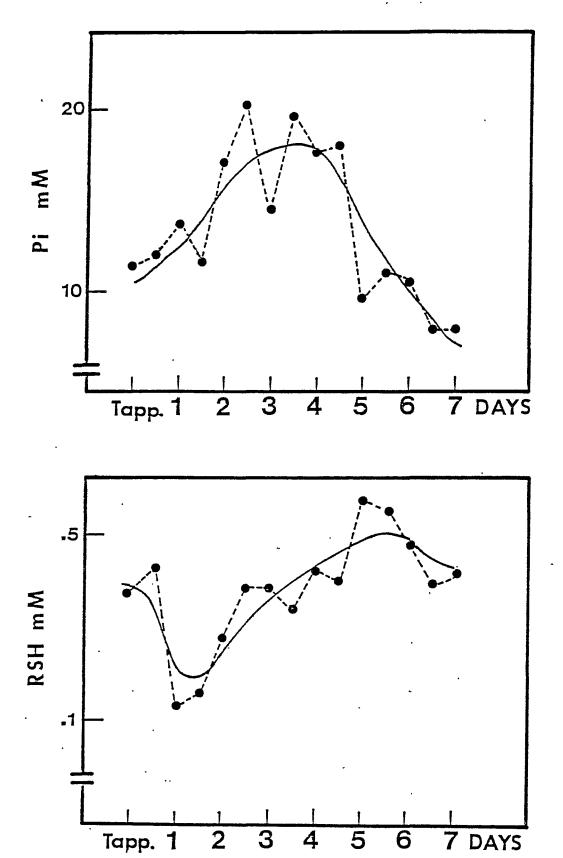
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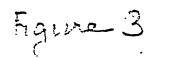
Figure 2

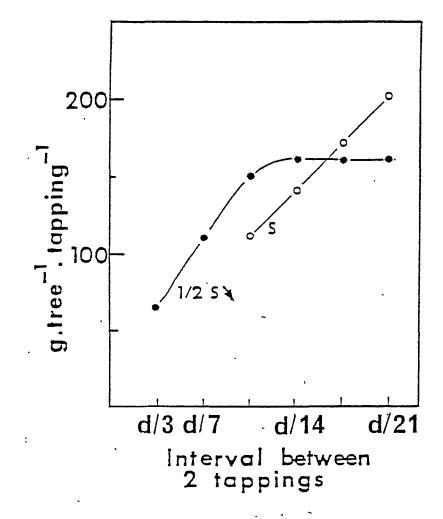
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